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(57) Abstract

Compositions and methods for sealing tissue and preventing post-surgical adhesions are provided. The inventive compositions comprise a collagen or gelatin component, one or more cross-linking agents, and optionally, a plasticizer. Methods for sealing tissue comprise applying the heated inventive compositions to tissue to form a continuous layer on the tissue and then allowing the sealant to cure to form a biocompatible layer that adheres to the tissue and degrades in at least about 2-120 days.

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COMPOSITIONS AND METHODS FOR SEALING TISSUE AND PREVENTING POST-SURGICAL ADHESIONS

TECHNICAL FIELD

The present invention relates generally to compositions and surgical methods for effecting and enhancing wound closure in tissue and compositions and methods for preventing post-surgical adhesions. More particularly, the present invention relates to compositions and methods for applying these compositions to tissue to close wounds and seal severed vessels and to provide a barrier between healing tissue planes.

BACKGROUND ART

Most surgical disciplines are concerned with the repair of damaged tissues and vessels. Tissue damage can be the result of direct trauma to the body or as part of a surgical procedure in which there is a separation of normally continuous tissue such as blood vessels. Historically, suturing has been the accepted technique for rejoining severed tissues and closing wounds. To suture a wound, the surgeon manually stitches the surrounding tissues with a surgical needle and suturing thread, and more recently, with a variety of polymeric or metallic staples.

While suturing and stapling techniques are often successful, there are a number of limitations inherent in such mechanical approaches. The practice of suturing or stapling tissue together not only requires significant skill, but is a relatively slow process, particularly when extensive repair is required or when anastomosing tiny biological structures. Even when suturing is properly performed, however, this technique can be less than satisfactory because of the gaps

which are left between the stitches and the possibility of progressive structural weakening over time. For example, the gaps leave the wound open to bacteria, producing a risk of infection. In addition, the suture needle or staples puncture the tissue, producing holes through which biological fluid may leak.

In an effort to overcome the difficulties associated with conventional suturing techniques, sutureless repairs using surgical adhesives or glues have been developed. These surgical adhesives adhere to tissue surfaces and form a bond until the tissue heals. For example, one common tissue adhesive comprises fibrin and typically contains fibrinogen and thrombin. These agents are mixed together to form an adhesive capable of joining separated tissues. Fibrin adhesive, however, is usually obtained from pooled human plasma and the threat of infection from agents such as Hepatitis "B", HIV or others has outweighed the benefits of obtaining commercial quantities of fibrin adhesive. In addition, other disadvantages of fibrin adhesives include its relatively slow set up time (>5 minutes).

Non-biological materials, such as cyanoacrylates including isobutyl-2-cyanoacrylate, have also been examined as potential surgical adhesives. These materials, however, are generally irritating to tissues, difficult to apply because they require a dry field and often fail to form a permanent closure. (Tatooles *et al.* (1966) *Surgery* **50**(4):857-861).

Glues based on gelatin-resorcinol crosslinked with formaldehyde have been used experimentally. Several formulations are known of which gelatin-resorcinol-formaldehyde "GRF" is best known (Braunwald *et al.* (1966) *Surgery* **59**(6):1024-1030). Warm solutions of gelatin-resorcinol are mixed in situ with a curing agent consisting primarily of a formaldehyde solution. The sealant rapidly sets to a solid which adheres to tissue. The main disadvantage has been the required use of both formaldehyde and resorcinol, known hazardous materials. (Tatooles, *et al.*, *supra*).

Because of the known toxicities of formaldehyde and resorcinol, Ennker et al. evaluated substitute crosslinking agents. (Ennker *et al.* (1994) *Ann. Thoracic Surgeons* **57**:1622-1627). Pentane 1,5 dial (glutaraldehyde) and ethanedial in combination with gelatin-resorcinol were evaluated and found to be less toxic than formaldehyde. However, Ennker also teaches that extensive toxicity studies including resorption kinetics will be necessary to determine utility. In addition, the compositions taught by Ennker continue to contain resorcinol, a known hazardous material.

Gelatin-based adhesive agents have been used in a non-biological setting as book-binding agents (see Japanese patent (Kokai) No. 3-239781). The disclosed book-binding agent consists of a "glue-gelatin" that comprises a principal agent and a setting agent. The principal agent is described as either a gelatin-based material, vinyl acetate resin, ethylene resin, acrylic resin or acrylic-styrene resin. The setting agent is described as an aldehyde compound, tannin, epoxy compound, isocyanate compound or alum containing trivalent chromium and zirconium salts.

The combination of human serum albumin (HSA) or bovine serum albumin (BSA) and glutaraldehyde has been evaluated as a tissue adhesive (see US patent No. 5,385,606). However, although albumin may have an acceptable "glue-like" texture, it is not a structural protein and, upon application, may not maintain integrity or to adhere to underlying tissue. The use of BSA is also problematic because of its known antigenic properties.

An adhesive composition comprising non-crosslinked collagen or gelatin mixed with collagen crosslinked by either UV or thermal means is also disclosed for wound closure (EP 466383). The composition is deficient as an adhesive because the integrity of the bond is relatively low and the sealant, upon insertion into the body cavity, will disintegrate rapidly prior to wound healing.

With any surgical procedure, there is a risk that post-surgical adhesions may form. Post-surgical adhesion formation occurs frequently and may contribute to or cause compromised

surgical results and post-surgical complications. Surgical adhesions may occur in various areas: pelvic, abdominal, spinal, tendon, ophthalmic, urinary, thoracic and cardiovascular, and are formed when normal tissue bonds to the surfaces of internal organs which have been traumatized or damaged during surgery. Such adhesions may join organs or other body tissues that normally are separate. Treating adhesions may necessitate additional surgery with additional costs, danger and/or discomfort to the patient.

Several previous attempts have been made to minimize or eliminate post-surgical adhesions. These attempts include introducing blocking materials or barrier films, for example metals, synthetic polymers and natural materials, into the wound. A woven material of regenerated cellulose is currently marketed for this purpose by Johnson & Johnson under the registered trademark, Interceed. This cellulose product is, however, incapable of being immobilized onto the target tissue and remains unfixed *in vivo*.

Other polymeric materials that have been tried for this purpose include nylon, cellophane, PTFE, polyethylene, siloxane, elastomers and polylactic acid copolymer films. Many of these materials are not biodegradable and, therefore, remain in the body with unpredictable and undesirable consequences.

WO 9515747 discloses a method of preventing adhesions by topical administration of fibrinolysis enhancing agents, especially urokinase. However, the addition of pharmaceutical agents may adversely affect wound healing at the surgical site.

Other barrier devices have been used to prevent post-operative adhesions including sheets of a warped knit fabric of oxidized regenerated cellulose (Interceed, see US patent No. 5,007,916 and US patent No. 5,134,229); sheets of expanded polytetrafluoroethylene (Gore-Tex®) (Boyers *et al.* (1988) *Fertility and Sterility* **49**:1066-1070), thermoreversible hydrogels (US patent No. 5,126,141) and photopolymerized, resorbable hydrogels (US patent No. 5,468,505). None of these devices provide a means of preventing post-surgical adhesions

with a material that is capable of adhering to the underlying tissue, is biocompatible and, preferably, is biodegradable over a period of about 2-120 days, preferably over a period of about 2-90 days and more preferably over a period of about 2-30 days.

WO 9221354 describes the use of dextran sulfate, with a sulfur content greater than 10% by weight, for inhibiting fibrosis, granulation or scar formation of a surgical lesion. This material does not adhere to the underlying tissue.

The use of hyaluronic acid (HA) is also well known as a preventive agent for surgical adhesions. US patent No. 5,017,229 describes a novel composition comprising HA, a polyanionic polysaccharide like carboxymethylcellulose (CMC) and an activating agent to prevent surgical adhesions. This method also has limited success because the HA composite material is not secured to the underlying tissue and, therefore, does not provide an immobilized barrier between the tissues.

WO 86/00912 describes a slowly-degradable gel for preventing tissue adhesions following surgery prepared by cross-linking a carboxy-containing polysaccharide with a bi- or poly-functional epoxide. This gel does not adhere to the underlying tissue. US patent No. 5,135,751 discloses an aqueous composition for reducing post-surgical formation comprising a polyoxyalkalene block copolymer. US patents Nos. 5,080,893, 5,140,016 and 5,350,173 describe methods of preventing adhesions during surgery by applying a hydrophilic, polymeric substance prior to surgery. None of these methods provide a means for securing the material to the tissue to insure lasting separation of the tissue planes.

For these and other reasons, it would be desirable to provide compositions and methods for effectively sealing damaged tissue structures, such as torn vessels or open wounds. These compositions and methods should be capable of forming an immediate closure of the damaged tissue to prevent further gas, fluid or blood leakage, and creating a permanent seal around the wound, while minimizing damage or destruction of surrounding tissue. The compositions and methods should

also minimize or prevent the occurrence of post-surgical adhesions.

DISCLOSURE OF THE INVENTION

The present invention relates to compositions and methods of sealing tissue and preventing post-surgical adhesions comprising preparing a composition comprising a solution or suspension of a proteinaceous material such as collagen or gelatin and a solution of one or more protein crosslinking agents, optionally comprising a plasticizer, that has been heated to an elevated temperature to form a flowable sealant; applying the flowable sealant to the tissue to form a continuous layer of the sealant on the tissue; allowing the layer to continue crosslinking on the tissue to form a cured layer of the proteinaceous material adhered to the underlying tissue that degrades in at least about 2-120 days, preferably 2-90 days, more preferably 2-30 days. The proteinaceous solution or suspension preferably consists of about 5-60%, more preferably about 30-55%, even more preferably 40-50% collagen or gelatin. If the sealant is applied by spraying, the concentration of the proteinaceous material is preferably about 10-40%, more preferably about 25-35%. The crosslinking agent(s) are preferably aldehydes, preferably di- or polyaldehydes, more preferably a glutaraldehyde, glyoxal or a dialdehyde starch. Crosslinking agents also comprise oxidized carbohydrates, preferably malto-dextrins. Crosslinking agents also comprise epoxy compounds, preferably polymers with 2 or more pendant epoxy groups, more preferably a polyethylene glycol ether or a diglycidyl ether. The crosslinking agents effect a complete cure of the sealant in about 30 seconds to about 24 hours, preferably less than 5 minutes. The crosslinking agent(s) is present in an amount of about 0.01-35%, preferably 0.1-5%, more preferably 0.6-1.2%. The plasticizers comprise polymers, preferably polyethylene glycol having a molecular weight of 200 to 1000 Daltons, more preferably 400 Daltons. Plasticizers are present in an amount of about 1-30%, preferably 5-10%.

BRIEF DESCRIPTION OF THE DRAWINGS

Figure 1 shows the viscosity of gelatin solutions at various temperatures. For example, the plot for the 50% gelatin solution shows that the viscosity of the material is approximately 30,000 centipose (cP) at 50°C. Viscosity measurements for more dilute solutions, e.g., concentrations which are appropriate for spray delivery, are also shown.

Figure 2 shows the change in elastic modulus of an adhesive formulation over various cure times. 4 parts of 50% (w/v) gelatin in 0.2 M sodium phosphate, pH 8.0, were mixed with 1 part of 3% (w/v) dialdehyde starch in water. After adding dialdehyde starch, the viscosity initially decreases due to dilution of the gelatin solution. As the dialdehyde starch crosslinks the gelatin, the solution becomes a gel due to the formation of Schiff bases between the oxidized starch and amino groups on gelatin. The strength of the gel may then be measured by elastic modulus as shown. Also shown is 50% gelatin mixed with water in a 4:1 ratio, demonstrating that the addition of dialdehyde starch hastens curing of the gelatin and strengthens the final sealant composition.

Figure 3 illustrates the behavior of the cured sealant composition in physiological saline solution at 37°C. Gelatin (50% aq. solution) and dialdehyde starch (3% aq. solution) were mixed in a 4:1 ratio to form a rubbery solid. This rubbery substance remained intact for more than 1 week and then slowly disintegrated. Gelatin mixed with water in the same manner dissolved in approximately 1 day. These results simulate the time course of persistence of the gel *in vivo* and demonstrate the stability of the crosslinked gelatin, compared to gelatin alone.

BEST MODE FOR CARRYING OUT OF THE INVENTION

The present invention relates generally to compositions and surgical methods for effecting and enhancing wound closure in tissue and compositions and methods for preventing post-surgical adhesions. More particularly, the present invention relates to a composition comprising a sealant which is heated in order to allow it to be applied as a flowable substance to

close wounds and seal severed vessels and methods of sealing tissue utilizing the composition.

The heated sealant comprises two or more components; the required components are a proteinaceous material such as collagen or gelatin, preferably gelatin, and a protein crosslinking agent. The crosslinking agent is preferably an aldehyde, more preferably, a di-or polyaldehyde, even more preferably di-aldehyde starch or glutaraldehyde. The sealant may also comprise a plasticizer. The plasticizer increases the malleability, flexibility and the rate of degradation of the sealant. Preferably the plasticizer is an alcohol, more preferably a polyethylene glycol (PEG), more preferably PEG with a molecular weight ranging from about 200 to about 1,000 Daltons, more preferably about 400 Daltons.

Compositions of the sealant are generally prepared as follows. A sample of at least about 5-60% (w/w) solution or suspension of a proteinaceous material such as collagen or gelatin and at least about 0.01-35.0% of a protein crosslinking agent are mixed together to form a crosslinked, cured sealant. The composition may include a plasticizer, such as PEG, in an aqueous solution or suspension of at least about 1-30%, preferably 5-10%. A plasticizer may be added to the proteinaceous material, the crosslinker or both. The composition may also contain a viscosity enhancer such as a biocompatible polymer or sugar, such as maltose, glycol or PEG. The compositions may also contain a solvent such as water, dilute buffered saline solution, phosphate buffer and the like.

As one skilled in the art will appreciate, compositions within the present invention can be made using protocols that are within the present invention but differ somewhat from those described herein. To prepare an inventive composition, a sample of proteinaceous material such as gelatin or collagen in a phosphate or other biocompatible buffer is combined with a protein crosslinking agent. The pH of the gelatin solution or suspension is related to the crosslinking agent employed. For example, crosslinking with Schiff base formation, *i.e.*, with aldehydes, requires a pH from 6.0-11.0, preferably 7.0-

9.0. Alternatively, crosslinking with other agents will require different pH ranges which can be determined without undue experimentation by one of skill in the art.

Compositions of the present invention bond to various organs and tissue, such as muscle, skin, epithelial tissue, connective or supporting tissue, nerve tissue, ophthalmic and other sense organ tissue, vascular and cardiac tissues, gastrointestinal organs and tissue, pleura and other pulmonary tissue, kidney, endocrine glands, male and female reproductive organs, adipose tissue, liver, pancreas, lymph, cartilage, bone, oral tissue, mucosal tissue and the like.

The sealants may also provide strong and rapid bonding to either natural or synthetic materials, including Dacron®, nylon, polypropylene and silicone in various forms including mesh. The sealants may also bond to Teflon®-coated materials, cellulose materials such as paper, cardboard and the like and metals including stainless steel, titanium and the like. Compositions of the present invention are also useful for sealing tissue by attachment of a patch as described in co-owned and co-pending applications, U.S. serial nos. 08/461,227, 08/461,228, 08/481,712, 08/303,336, 60/006,321, 60/006,322 and 60/006,324. Composition of the present invention may also be useful in the attachment of surgical devices and prosthetics as well as for wound closure, trauma repair and the like.

When employed as surgical adhesives, compositions of the present invention are flowable at the time of delivery and have a peel bond strength to the underlying tissue of at least about 3.0 N/m. Peel bond strengths higher than about 3.0 N/m are acceptable. Compositions of the present invention are biocompatible and remain immobile until the tissue is satisfactorily sealed and preferably will resorb by biodegrading in about 2-120 days, preferably 2-90 days, more preferably 2-30 days following application. Compositions of the present invention also prevent post-surgical adhesions by providing a barrier between opposing healing tissue planes.

Compositions of the present invention may be applied to tissue in any "flowable" state including a gel, paste, foam,

sol, liquid, spray or the like. If the sealant composition is sprayed it may be sprayed as an aerosol and the like. The concentration of the components of the inventive compositions will vary depending upon the preferred form of delivery. One skilled in the art will be readily able to determine from the formulations of the compositions disclosed herein the concentrations of the various components of the inventive compositions necessary to achieve the desired consistency.

Upon contacting the tissue, the sealant should be sufficiently fluid to flow into surface tissue irregularities. This provides good apposition of tissue and sealant. Viscosities less than about 80,000 cP are adequate, however, a viscosity of less than about 20,000 cP is preferable, and a viscosity of less than about 4,000 cP is even more preferable. In addition to the need for the sealant to be fluid upon application, the sealant must also be of a sufficient viscosity to coat the desired tissue site but not so fluid as to flow away from the desired tissue site to other tissue regions. To maintain placement of the sealant, the minimum viscosity must be at least 1000 cP, and more preferably, 2000-4000 cP. The desired viscosity of the sealant is related to the time required for curing. For example, the viscosity of a fast-curing sealant can be lower than that of a slow-curing sealant because rapid curing will cause the sealant to remain in place.

If the sealant is applied as a spray or foam, lower viscosities may be preferred; however, a minimum peel bond strength to the underlying tissue of at least about 3 N/m is required. In addition, a minimum elastic modulus of about $1x10^5$ N/m^2 is also required in order for the sealant to provide adequate closure of the wound, incision or defect and form an immobilized continuous barrier layer to prevent post-operative adhesions.

Such formulations are particularly useful for wound closure. Upon mixing, the sealant components crosslink to become an elastic solid that is very soft initially, but within minutes becomes a non-tacky, rubbery elastic solid useful for closing various forms of wounds including surgical

incisions, wounds caused by trauma and other defects. The sealant composition, in addition to its utility for wound closure also provides a method to prevent and/or reduce post-operative adhesions.

The superior results obtained by the compositions of the present invention are noted in Figures 2 and 3. These figures illustrate that the addition of a protein crosslinking agent to collagen or gelatin produced far better results that those obtained with gelatin alone. The superior qualities of the inventive compositions include an ability to survive well past 200 hours (greater than 8 days) and a greater bond strength when compared to a single component, gelatin sealant. Compositions within the present invention are superior sealants because they exhibit excellent peel bond strength, elastic modulus, survival (persistence), are biocompatible and also prevent or reduce the occurrence of post-operative adhesions.

Exemplary delivery systems that may be used in conjunction with compositions within the present invention include the following criteria: a) a cartridge to hold and dispense sealant. The cartridge may have a single or double barrel, available from Mixpac; Conprotec, Inc., Salem, NH and the like; b) a pump or dispense actuation mechanism such as a pressure pump available from Johnson Medical Devices, Milford, NH, or a pneumatic dispense pump available from I.J. Fisnar, Fairlawn, NJ, and the like; c) a dispensing nozzle capable of mixing and dispensing sealant to the target site; and d) a heating element that may be incorporated as a part of the apparatus or may stand alone such as a heating block or water bath.

As used herein, "proteinaceous material" includes fibrin, fibrinogen, thrombin, albumin of human or animal origin, Factor VIII and elastin.

As used herein, "collagen" includes various types of native and denatured collagen including type I collagen, type III collagen, type IV collagen, collagen rich tissue, atelopeptide collagen and the like , or a combination thereof. Bovine, porcine or any other appropriate vertebrate collagen

are all exemplary tissue sources of collagen. One skilled in the art can readily determine the necessary adjustment in the bloom of the collagen which varies among the commercial sources of collagen.

As used herein, "gelatin" is the well-known denatured form of collagen as defined above. Gelatin may be produced from bovine or porcine hide or bone, or may be produced by denaturing collagen using heat or other methods known in the art.

As used herein, "biocompatible" means the sealant composition of the present invention meets the criteria outlined in the current North American Science Associates, Inc. (NAmSA®) guidelines for evaluating biomaterial and devices as defined by committees of the Tripartite Biocompatibility Guidance and the International Organization for Standardization (ISO) (NAmSA, Northwood, OH)

As used herein, "protein crosslinking agent" means an agent that is capable of bonding or causing bonds to form between at least two functional groups of a protein. Exemplary crosslinking agents include, but are not limited to: epoxy compounds, such as polymers with 2 or more pendant epoxy groups, polyethylene glycol di-glycidyl ethers, preferably having a molecular weight of about 200 to about 3400D; N-hydroxysuccinimidyl compounds, such as di-N-hydroxy-succinimidyl-polyethylene glycol, preferably having a molecular weight. of about 200 to about 3400D and di-N-hydroxy-succinimidyl-suberate; carbodiimide compounds, such as 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide; imidate compounds, such as dimethylsuberimidate; carbonyl-imidazoles, such as di-carbonyl-imidazole-polyethylene glycol, molecular weight. 200-3400D; tresylates, -OSO2CH2CF3, such as di-tresyl-polyethylene glycol, molecular weight. 200-3400D; di-isocyanates, such as hexamethylene-diisocyanate; acid anhydride compounds, such as co-polymers of polyethylene oxide allyl ether and maleic acid anhydride; di-cyanuric chloride compounds, such as di-cyanuric chloride derivatives of polyethylene glycol, molecular weight. of at least about 200 to about 3400D; bisiodoacetamide, such as N,N' ethylene

bisiodoacetamide; methylene bibromide and related compounds; hydrazine and related compounds, oxidized carbohydrates such as maltodextrins, polysaccharides such as carboxymethyl cellulose and the like and combinations thereof.

Aldehydes obtained by oxidative cleavage of carbohydrates and their derivatives, such as oxidized oligosaccharides, *e.g.*, malto-dextrins and cellulose derivatives of various molecular weights are preferred crosslinking agents, while poly- or di-aldehydes are more preferable and dialdehyde starch is even more preferable.

As used herein, "elevated temperature" means a temperature at which the protein component of the sealant remains in a fluid state, but that does not cause an extensive cauterizing or damaging effect to the underlying tissue. Exemplary temperatures are about 50-80°C. The sealant may be heated to higher temperatures, however, at delivery, the sealant is preferably at a temperature of about 50 to about 70°C when it contacts the tissue.

As used herein, "flowable" means a fluid state wherein the viscosity of the composition is about 80,000cP or less; preferably about 20,000 or less; more preferably about 2,000cP.

As used herein "cured" means sufficient cross-linking has occurred to achieve the desired mechanical strength and stability to dissolution at 37-40°C. Crosslinking may be measured by elastic modulus. A cured composition will exhibit a plateau elastic modulus of about $1X10^6$ N/m^2 and will remain solid or gelatinous (will not re-melt or dissolve) at 37-40°C.

As used herein, "continuous layer" means an uninterrupted, smooth, even surface layer that fills inconsistencies, crevices, divots and/or gaps in the underlying tissue. The amount of sealant required to achieve a continuous layer will depend on the type of underlying tissue and the pressure, if any, present in the organ being sealed.

As used herein, "adhere" means having a peel bond strength of at least about 3.0 N/m.

As used herein, "persistence" means the sealant remains intact and immobilized for at least about 2-120 days, preferably 2-90 days, and more preferably 2-30 days. Following such time period, the material will biodegrade.

As used herein, "intact" means that the sealant remains in one piece and does not disintegrate for at least about 2-120 days, preferably 2-90 days, and more preferably 2-30 days.

As used herein, "elastic modulus" means the stress divided by the strain. The "stress" and "strain" are defined in any text on rheology, such as VISCOELASTIC PROPERTIES OF POLYMERS, Ferry, J.D. (Wiley, New York, 1980) or Bird *et al.*, DYNAMICS OF POLYMERIC LIQUIDS (Wiley, New York, 1977).

The final components of the inventive compositions include an aqueous solution or suspension of at least about 5-60% proteinaceous material, preferably collagen or gelatin, preferably in the range of 30-55%, more preferably in the range of 40-50% or, if applied by spraying, in the range of 10-40%, preferably in the range of 25-35%, and at least about 0.01%-35% of a protein crosslinking agent, preferably in the range of 0.1- 5%, more preferably in the range of 0.6-1.2%. The gelatin or collagen and protein crosslinking agent are in a final weight ratio of 2:1 to 500:1. Various solvents may be used including water, dilute buffer saline solution, phosphate buffer and the like. The compositions of the present invention may also include a plasticizer such as PEG in a final amount of about 1-30%, preferably 5-10%. The molecular weight of the PEG may be in the range of about 200 to 1000 Daltons, preferably 400 Daltons.

The components of the inventive compositions may be combined after they are heated separately at 70°C. The sealant components may be heated prior to delivery, for example, in a heat block or hot water bath, and/or may be placed into a heated device for delivery, for example, an epoxy glue gun or heated dual syringe with a heat source. The material may be heated to about 50-100°C prior to application. However, upon application, the temperature of the sealant will rapidly decrease until reaching body or ambient temperature.

Upon mixing of the proteinaceous and crosslinking components, crosslinking immediately commences and continues until maximum crosslinking is achieved. The degree of the crosslinking achieved depends on the ratio of protein to crosslinker. The degree of crosslinking is measured by assaying for free functional groups on the protein or by elastic modulus (see Figure 2). Crosslinking of the sealant may occur over a period of about 30 seconds to about 24 hours at 37°C depending on the crosslinking agent used.

Exemplary embodiments of the inventive sealant may include mixtures of more than one crosslinking agent. The cure time for a "fast-curing" crosslinking agent is less than or equal to 5 minutes, while the cure time for a "slow-curing" crosslinker is from about 5 minutes to 24 hours. If two or more "fast-curing" agents are employed, the cure time will remain less than or equal to 5 minutes, but the resulting sealant will exhibit superior mechanical strength. If a "fast-curing" and a "slow-curing" agent are employed, the resulting sealant will exhibit superior strength and persistence.

A "fast-curing" crosslinking agent, for example, a dialdehyde, carbodiimide or di-isocyanate, is preferred for indications such as wound or incision closure or organ tissue repair that requires rapid curing of sealant to insure integrity of seal, *e.g.*, sealing air leaks in lung tissue such that gas, fluid or blood leakage is prevented. "Slow-curing" agents, such as epoxy compounds, are preferred for applications such as securing a prosthetic device, shunt or other implantable device. In certain indications, the combination of a slow-curing crosslinking agent and a fast-curing crosslinking agent may be preferred.

The sealant compositions described herein are also useful for the prevention or reduction of post-surgical adhesions. Methods for preventing post-surgical adhesions comprise coating tissue surfaces in a patient with the compositions described herein. Tissue surfaces can be coated either during or after a surgical procedure, but prior to final closure of the patient.

The sealant compositions may be placed on the target tissue as the final step of any surgical procedure, *i.e.*, immediately prior to closing the surgical wound. The sites furthest away from the surgical incision are treated with the sealant compositions first, followed by sites that are closer to the incision site. This will minimize excessive manipulation of the previously placed sealant material.

The sealant may be applied to any site that has been manipulated surgically or damaged by any other means, including but not limited to, areas such as suture or staple lines, areas of resection, areas of tissue denudation, areas of serosal damage, or areas where formation of adhesions is anticipated , such as sites of tissue trauma, areas of cautery or other types of manipulation.

The sealant is applied to areas that are dry or as free of pooled blood or fluid as possible. The site may be dried using cautery, direct pressure, suction or any other standard means.

Using various delivery systems as described herein, the sealant compositions should be applied in such a manner that the entire area of interest is covered with a continuous layer of the sealant composition. Accordingly the sealant should completely fill and coat any divots, creases, or other defects in the target tissue and should complete cover the entire defect. When applied correctly, the sealant compositions should extend at least 5 mm beyond the perimeter of the target area.

The sealant compositions described herein are also useful for local delivery of biologically active substances to tissue surfaces of a patient. Methods of local delivery of biological substances comprise the steps of mixing a biological substance with the inventive composition and then applying the resulting mixture to the target tissue. Biologically active substances include, but are not limited to, proteins, carbohydrates, nucleic acids, and inorganic and organic biologically active molecules such as enzymes, antibiotics, antineoplastic agents, local anesthetics, anti-inflammatory agents, hormones, antiangiogenic agents,

antibodies, neurotransmitters, psychoactive drugs, drugs affecting reproductive organs and oligonucleotides such as antisense oligonucleotides.

All references, patent applications and patents cited herein are hereby incorporated by reference.

The following examples are presented to describe preferred embodiments and utilities of the present invention and are not meant to limit the invention unless otherwise stated in the claims appended hereto.

Example 1 - Sealant Formulations prepared with Aldehydes

Sealant formulations prepared with glutaraldehyde:

A 2.0 mL aliquot of a 44.44% solution of gelatin (Kraft Foods, Inc., Woburn, MA) in 0.2 M phosphate buffer, pH 8.0, was prepared in 3 mL syringe. 0.6 mL aliquots of 0.25, 0.5 and 0.75% solutions of glutaraldehyde (J.T. Baker, Inc. , Phillipsberg, NJ) were prepared in 3 mL syringes. After heating the syringes separately at 70^0 C for 30 minutes, aliquots of the gelatin and glutaraldehyde were mixed by pushing the contents of one syringe into the other using a three-way stop cock and then extruded onto an inflated porcine lung *in vitro*. Each formulation exhibited excellent bonding and integrity. The peel bond strength to the underlying porcine lung tissue was approximately 100N/m or greater. After complete mixing, the relative percentages of each component of the sealant formulations were:

TABLE 1

Gelatin	Glutaraldehyde	Composition
2.0 mL of 44.44%	0.6 mL of 0.25%	Gelatin, 34.1% and gl.al, 0.05%
2.0 mL of 44.44%	0.6 mL of 0.50%	Gelatin, 34.1% and gl.al, 0.115%
2.0 mL of 44.44%	0.6 mL of 0.75%	Gelatin, 34.1% and gl.al, 0.173%

Sealant formulations prepared with formaldehyde:

In a method similar to that described above, 2.0 mL aliquots of a 44.44% solution of gelatin (Hormel Food Corporation, Davenport, IA) in 0.2 M phosphate buffer, pH 8.0, were crosslinked with 0.6 mL of 0.5, 0.75, 1.5 and 3% solutions of formaldehyde (J.T. Baker, Inc. , Phillipsberg, NJ)after heating separately for at 70^0 C for 30 minutes. Sealants crosslinked with formaldehyde generally exhibited a decrease in peel bond strength and integrity compared to sealants crosslinked with glutaraldehyde or dialdehyde starch. After complete mixing, the relative percentages of each component of the sealant formulations were:

TABLE 2

Gelatin	Formaldehyde	Composition
2.0 mL of 44.44%	0.6 mL of 0.50%	gelatin, 34.1% and for.ald, 0.115%
2.0 mL of 44.44%	0.6 mL of 0.75%	gelatin, 34.1% and for.ald, 0.173%
2.0 mL of 44.44%	0.6 mL of 1.50%	gelatin, 34.1% and for.ald, 0.346%
2.0 mL of 44.44%	0.6 mL of 3.00%	gelatin, 34.1% and for.ald, 0.692%

Sealant formulations prepared with dialdehyde starch:

In a method similar to that described above 2.0 mL aliquots of a 44.44% or 50% solution of gelatin (Atlantic Gelatin, Woburn, MA) in a 0.2 M phosphate buffer, of pH 8.0, were crosslinked with 0.55 mL of a 3% solution with degrees of oxidation of 75% and 98% of a dialdehyde starch prepared from potato starch by periodate oxidation, according, METHODS IN CARBOHYDRATE CHEMISTRY, Vol. 4, (Whistler, R., *et al.*, eds., 1964). after heating separately at 70^0 C for 30 minutes. After

complete mixing, the relative percentages of each component of the sealant formulations were:

TABLE 3

Gelatin	DAS	Composition
10.00 mL of 44%	2.5 mL of 3% solution with a degree of oxidation of 75%	35.52% gelatin and 0.6% DAS
10.00 mL of 50%	2.5 mL of 3% solution with a degree of oxidation of 75%	40.00% gelatin and 0.6% DAS
10.00 mL of 44%	2.5 mL of 3% solution with a degree of oxidation of 98%	35.52% gelatin and 0.6% DAS
10.00 mL of 50%	2.5 mL of 3% solution with a degree of oxidation of 98%	40.00% gelatin and 0.6% DAS
1.50 mL of 38.4% gelatin and 3.8% PEG400	0.4 mL of 2% solution with a degree of oxidation of 75%	30.36% gelatin, 3.00% PEG & 0.42% DAS

Example 2 - Sealant formulations prepared with non-aldehyde crosslinking agents:

1.5 mL aliquots of a 35% solution of gelatin (Hormel Food Corporation, Davenport, IA) in 0.2 M phosphate buffer, pH 8.0, were separately crosslinked with 0.4 mL of 50% PEG 600 diepoxide (Polysciences, Inc., Warrington, PA) in water; with 0.1g hexamethylene-diisocyanate (Aldrich, Milwaukee, WI) in 0.1g acetone; and with 0.53g of 10% solution of 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide (Sigma Chemical Co., St. Louis, MO) after heating separately at 70° C for 30 minutes. After complete mixing, the relative percentages of each component of the sealant formulations were:

TABLE 4

Gelatin	Crosslinker	Composition	Comments
1.5 mL of 35% gelatin in pH 8.0 phosphate	0.4 mL 50% PEG(600) diepoxide in water	27.60% gelatin & 10.50% epoxide	When mixed at 70° C and stored at 41° C, crosslinking occurs between 5 and 16 hrs. When mixed at 60° C and stored at 60° C, crosslinking occurs at about 3 hours.
1.5 mL of 35% gelatin in pH 9.0 phosphate buffer	0.1g hexamethylene-diisocyanate in 0.1 g acetone	30.88% gelatin & 5.88% diisocyanate	Instantaneous (< 1 min) crosslinking when mixed at 60° C.
1.5 mL of 35% gelatin in pH 7.5 phosphate buffer	0.53 g of 10% aq. sol- ution of EDC*	25.86% gelatin & 2.61% EDC	Crosslinks in 6 min at 41°C.

* EDC = [1-Ethyl-3(3-dimethylaminopropyl)carbodiimide.Hydrochloride]

Example 3- Wound Closure

A triangular incision, measuring 2.5mm x 2.5mm x 2.5mm, was made at the right caudo ventral tip of the right cranial lung lobe of a farm grade Hampshire/Yorkshire cross pig (Pork

Power Farms, Turlock, CA). Three bronchioles were exposed and were leaking air when ventilated at a pressure of 28 cm of water. A glue syringe, which had been pre-heated in a 70° C water bath for at least 30 minutes, was taken out of the bath and cleaned with gauze. The stopper was removed and a mixing nozzle affixed to the syringe. Approximately 4.5 g of sealant was extruded and applied to completely cover the defect and extend about 5 mm beyond the perimeter of the defect. After 2.5 minutes, the sealant was irrigated with saline and at 3.5 minutes it became non sticky to touch. When the applied sealant was grasped with forceps, it was a solid film that appeared to adhere quite well to the tissue and had good integrity. The sealant was challenged by ventilating the lung to a pressure of 28 cm water. No air leak was noted. The chest incision was then closed in a routine fashion by suturing and air flow from the chest cavity (leakage from the surgery site) monitored. Air flow reached a zero flow rate within about 60 seconds and was maintained over a three hour period.

The delivery system used was a 4:1 double-barrel epoxy gun (Mixpac, Conprotec, Salem, NH). It contained a 50% (wt/wt) gelatin solution in 0.2M phosphate buffer, pH 8, in the larger barrel and a 3% (wt/wt) aqueous solution of DAS with a degree of oxidation of 75% in the smaller barrel. The composition of the sealant after mixing and as applied was 40 % gelatin and 0.6% DAS in phosphate buffer. The loaded dual barrel syringe was warmed in a water bath at 70°C for at least 30 minutes before use.

The animal model was allowed to recover for 7 days post-surgically. Recovery was uneventful and the animal exhibited no post-operative complications during recovery. After 7 days, the animal was euthanized and a necropsy was performed. The operative site was healing well. The sealant was intact at the site of application. There was vascular in-growth and healing evident at the site of the sealant. No post-operative adhesions were observed at the site of the sealant on the lung incision.

Example 4- Preparation of a gelatin sealant

A gelatin formulation of the present invention was initially prepared as a 50% (wt/wt) gelatin solution which was then mixed in a 4:1 ratio with the aqueous 3% DAS solution described in Example 3. Thus, after mixing, the original 50% solution was diluted to an approximate concentration of 40% gelatin and then applied to the tissue. The sealant was dispensed as a flowable mixture at 70°C and cooled to physiological temperatures as it cured. Figure 1 shows the viscosity of gelatin solutions at various concentrations as a function of temperature.

Example 5- Viscosity Measurement

Viscosity measurements (see Figure 1) were used to determine concentrations of sealant components suitable for application. Preferred concentrations for applications of the collagen or gelatin component of the sealant by spraying or painting were determined to be in the range of about 5 to about 30%.

Example 6- Peel Bond Measurement

2.5 mL of the sealant prepared according to the method of Example 1 using 50% gelatin and 3% dialdehyde starch was extruded *in vitro* onto a porcine lung inflated to about 15-25 cm water hydrostatic pressure, heated to about 30-40°C and irrigated with normal saline. After 3 minutes, the applied sealant was rubbery an non-tacky. A plastic tab, 1cm X 3 cm, was bonded to the sealant surface with cyanoacrylate (Super Glue®). The upper edge of the tab was perforated and a hook was affixed through this perforation. The hook was connected to a hand-held Omega DFG51-2 digital force gauge (Omega Engineering, Stamford, CT). The gauge was used to lift the sealant off the underlying tissue. One edge was separated to establish a peel line. The peel force as Newtons per length of peel line (in meters) is then estimated.

Example 7 - Sealant *In Vitro* Stability

1.0 mL gelatin, 300 Bloom, 50% (w/v) in 0.2M sodium phosphate, pH 8, was heated to 65-70°C for 10-30 minutes, and then mixed rapidly with 0.25 mL of 3% (w/v) aq. di-aldehyde starch DAS (75% degree of oxidation) prepared from potato starch by periodate oxidation, according to METHODS IN CARBOHYDRATE CHEMISTRY, Vol. 4 (Whistler, R., et al., eds., 1964). The mixture was extruded into tared vials at 37°C, incubated for 10 minutes, and then covered with a solution of 0.9% sodium chloride and held at 37°C. At intervals, the buffer was poured off the gel, and excess buffer drained from the gel with an absorbent tissue. The vial and gel were weighed. The gel weight was a measure of gel plus absorbed buffer. At later time points, the gel began to disintegrate, which was indicated by decreasing wet mass. The persistence of the gel in saline at physiological temperature was a simulation of behavior *in vivo*. In addition, a gel which did not dissolve for several days was evidence of covalent crosslinking between gelatin chains, since gelatin alone persisted for less than 30 hours in this assay (* and ●). In Figure 3, 50% gelatin was reacted with 3% DAS in two samples (■ and ▲). Two additional samples were prepared from 50% gelatin and 3% DAS to which glutaraldehyde (GA) was added at 0.02% (w/v), to yield 0.005% GA in the final gel mixture (◆ and X). Under these conditions, with relatively low levels of added glutaraldehyde, glutaraldehyde did not increase the persistence of the gel mass.

Example 8 - Elastic Modulus

Figure 2 illustrates the curing of the sealant by measuring its elastic modulus as defined above. As the sealant was curing to become an elastic solid, increasing modulus values were observed and on completion of curing, the elastic modulus remained constant. Measurements of the elastic modulus as shown in Figure 2 were made by measuring the force required to compress a curing sealant under small strain, *i.e.*, strains less than 0.1. The formulation of the sealant was approximately 2mL of 50% (w/w) Woburn gelatin in

0.2 M phosphate buffer , pH 8.0, mixed with 0.6mL of 3% DAS oxidized to 75%. The sealant was mixed at 70°C and maintained at 37°C for the duration of the curing process. The mixed sealant was placed in a 3cc syringe and a rigid plastic plunger was used to compress the sealant. Compressional forces were measured using a digital force gauge and strains were measured on a digital displacement stand. Figure 2 demonstrates that the sealant began curing immediately and was completely cured within 12 minutes (●). Gelatin alone (▲), without dialdehyde starch, also gelled at 37°C, but the gel strength (modulus) was less than that of gelatin crosslinked with dialdehyde starch.

EXAMPLE 9 - Antiadhesion Animal Study

A standard animal model for evaluating surgical adhesions has been developed using the Sprague Dawley rat (Harris, E.S. (1995) "Analysis of the kinetics of peritoneal adhesion formation in the rat and evaluation of potential anti-adhesive agents," *Surgery* **117**:663-669). In this model, a single, specific adhesion may be objectively measured. Anesthesia was induced with an intramuscular injection of ketamine hydrochloride in combination with xylazine in 21 animals. The abdomen was clipped with electric clippers and the skin prepared with an iodophor scrub and rinsed with 70% alcohol. A 6 cm midline skin incision was made at the abdomen, and the skin retracted. A 4 cm incision was made through the abdominal wall. The abdominal wall was also reflected. A defect in the abdominal wall was created approximately 1 cm lateral to the midline incision. This defect was created by excising a 1 x 2 cm segment of parietal peritoneum, including a superficial layer of muscle. The cecum was then elevated and positioned so that at closure, the cecum contacted the abdominal wall defect. A 1 x 2 cm defect was then created on the serosal surface of the cecum. The cecum was abraded by scraping with a scalpel blade so that a homogenous surface of petechial hemorrhage was created over the abraded area. Both areas of defect (the cecum and body wall) were exposed to air

for 10 minutes. The animals were grouped for further manipulation as follows:

GROUP 1 CONTROL ANIMALS n = 5

The defects (abdominal wall and cecal) were allowed to air dry for ten minutes. Both lesions were then placed in contact, and the midline incision closed with a 4-0 absorbable suture in a continuous pattern. The skin was closed with a 9 mm Autoclip®.

GROUP 2 SEALANT "A" ON WALL DEFECT n = 5

Under sterile conditions, Sealant A (a final composition of 31.58% Gelatin mixed with 0.42% DAS) was applied. After air drying for 10 minutes, the abdominal wall defect was covered with Sealant A and allowed to cure for approximately 4 minutes. The sealant-covered peritoneal defect was placed into contact with the abraded, air-dried cecum, and the midline incision closed with a 4-0 absorbable Maxon® suture in a continuous pattern. The skin was closed with a 9 mm Autoclip®.

GROUP 3 SEALANT COMPOSITION "B" ON WALL DEFECT n = 5

Under sterile conditions, Sealant B (a final composition of 30.37% Gelatin mixed with 0.42% DAS and 3% PEG400) was applied. After air drying for ten minutes, the abdominal wall defect was covered with Sealant B and allowed to cure for approximately 4 minutes. The sealant-covered peritoneal defect was placed into contact with the abraded, air-dried abdominal wall defect, and the midline incision was closed with a 4-0 absorbable suture in a continuous pattern. The skin was closed with 4-0 absorbable Maxon® suture in a continuous pattern. The skin was closed with a 9 mm Autoclip®.

GROUP 4 SEALANT "C" ON CECAL DEFECT n = 5

Under sterile conditions, Sealant C (a final composition of 21.5% gelatin mixed with 1.5% DAS) was applied. After air drying for ten minutes, the cecal defect was covered with Sealant C and allowed to cure for approximately 4 minutes. The sealant-covered peritoneal defect was placed into contact with the abraded, air-dried cecum, and the midline incision

was closed with a 4-0 absorbable Maxon® suture in a continuous pattern. The skin was closed with a 9 mm Autoclip®.

GROUP 5 SEALANT "D" ON WALL DEFECT n = 1

Under sterile conditions, Sealant D (a final composition of 32.0% gelatin mixed with 0.42% DAS) was applied. After air drying for ten minutes, the abdominal wall defect was covered with Sealant D and allowed to cure for approximately 4 minutes. The sealant-covered peritoneal defect was placed into contact with the abraded, air-dried abdominal wall defect, and the midline incision was closed with a 4-0 absorbable Maxon® suture in a continuous pattern. The skin was closed with a 9 mm Autoclip®.

The results of this study are summarized in Table 5 below.

TABLE 5

SEALANT	FINAL (POST-MIXING) COMPOSITION	TISSUE TYPE	AMOUNT APPLIED	TIME TO CURE	ADHESION FORMATION OBSERVED
A (34-58-1)	GELATIN, 31.58 %, DAS, 0.42%	BODY WALL	1.5-2.0g	4 MIN	-
B (34-58-1)	GELATIN, 30.37 %, DAS, 0.42% , and PEG400, 3.0%	BODY WALL	1.5-2.0g	4 MIN	<
B (34-58-2)	GELATIN, 21.5% DAS, 1.5%	CECUM	1.5-2.0g	4 MIN	-
B (34-58-2)	GELATIN, 32.0% DAS, 0.42%	BODY WALL	1.5-2.0g	4 MIN	-
CONTROL	NONE				+

- = no adhesions
< = reduced adhesions
+ = adhesions formed (within control group, 4 of 5 animals formed adhesions)

Example 10 - Evaluation of Various Sealant Compositions

Using a farm grade Hampshire/Yorkshire cross pig (Pork Power Farms, Turlock, CA) various sealant compositions were

applied during non-survival surgeries to evaluate adherence and bonding of the sealant compositions to the underlying tissue. The results are shown in Table 6.

TABLE 6

	FINAL (POST-MIXING) COMPOSITION	TISSUE TYPE	AMOUNT APPLIED IN GRAMS	TIME TO CURE IN MINUTES	ADHERED TO UNDERLYING TISSUE?
1	GELATIN, 40.00%, DAS, 0.6%	LUNG	4.0-5.0	3.0	YES
2	GELATIN, 40.00%, DAS, 0.6%	LUNG	4.0-5.0	5.0	YES
3	GELATIN, 40.00%, DAS, 0.6%	AORTA	4.0-5.0	3.0	YES
4	GELATIN, 40.00%, DAS, 0.6%	BRONCHI	4.0-5.0	3.0	YES
5	GELATIN, 40.00%, DAS, 0.6%	INTESTINAL SURFACE	4.0-5.0	3.0	YES
6	GELATIN, 40.00%, DAS, 0.6%	LIVER	4.0-5.0	3.0	YES
7	GELATIN, 40.00%, DAS, 1.2%	COLON	4.0-5.0	3.0	YES
8	GELATIN, 40.00%, DAS, 1.2%	SPLEEN	4.0-5.0	3.0	YES
9	GELATIN, 40.00%, DAS, 1.2%	LUNG	4.0-5.0	5.0	YES
10	GELATIN, 40.00%, GLUT, 0.10%	LUNG	4.0-5.0	3.0-5.0	YES
11	GELATIN, 40.00%, GLUT, 0.10%	LUNG	4.0-5.0	3.0-5.0	YES
12	GELATIN, 40.00%, GLUT, 0.05%	KIDNEY	4.0-5.0	not recorded	YES
13	GELATIN, 40.00%, DAS 0.545, GLUT, 0..005%	LUNG	4.0-5.0	3.0	YES
14	GELATIN, 40.00%, DAS 0.568, GLUT, 0..0025%	LUNG	4.0-5.0	not recorded	YES
15	GELATIN, 33.33%, DAS, 1.00%	AORTA	4.0-5.0	not recorded	YES
16	GELATIN, 33.33%, DAS, 1.00%	LUNG	4.0-5.0	not recorded	YES
17	GELATIN, 34.82%, DAS, 0.66%	LUNG	4.0-5.0	not recorded	YES
18	GELATIN, 27.44%, DAS, 0.43%	LUNG	4.0-5.0	not recorded	YES
19	GELATIN, 27.44%, DAS, 0.66%	LUNG	4.0-5.0	not recorded	YES

WHAT IS CLAIMED IS:

1. A method of sealing tissue comprising:

a) mixing a solution or suspension of a proteinaceous material with a solution of a protein crosslinking agent at an elevated temperature to form a flowable sealant;

b) applying said flowable sealant to the tissue to form a continuous layer of said sealant on the tissue;

c) allowing the sealant to continue crosslinking on the tissue to form a cured biocompatible layer adhered to the tissue that degrades in at least about 2-120 days.

2. The method of claim 1, wherein the cured layer degrades in at least about 2 - 90 days.

3. The method of claim 1 wherein the cured layer degrades in at least about 2-30 days.

4. The method of claim 1, wherein the proteinaceous material is collagen or gelatin.

5. The method of claim 4, wherein the solution or suspension consists of about 5% to about 60% collagen or gelatin.

6. The method of claim 5, wherein the solution or suspension consists of about 30% to about 55% collagen or gelatin.

7. The method of claim 6, wherein the solution or suspension consists of about 40% to about 50% collagen or gelatin.

8. The method of claim 4, wherein the solution or suspension is about 10% to about 40% collagen or gelatin and the sealant is applied to the tissue by spraying.

9. The method of claim 8, wherein the solution or suspension is about 25% to about 35% collagen or gelatin and the sealant is applied to the tissue by spraying.

10. The method of claim 1, wherein the sealant further comprises a plasticizer.

11. The method of claim 10, wherein the plasticizer is polyethylene glycol having a molecular weight ranging from about 200 to about 1000 Daltons.

12. The method of claim 11, wherein the plasticizer is polyethylene glycol having a molecular weight of about 400 Daltons.

13. The method of claim 10, wherein the plasticizer is present in an amount of about 1-30%.

14. The method of claim 13, wherein the plasticizer is present in an amount of about 5-10%.

15. The method of claim 1, wherein the crosslinking agent is an aldehyde.

16. The method of claim 15, wherein the aldehyde is a di- or polyaldehyde.

17. The method of claim 16, wherein the di- or polyaldehyde is glutaraldehyde, glyoxal, or dialdehyde starch.

18. The method of claim 1, wherein the crosslinking agent is an oxidized carbohydrate.

19. The method of claim 18, wherein the oxidized carbohydrate is malto-dextrin.

20. The method of claim 1, wherein the crosslinking agent is an oxidized polysaccharide.

21. The method of claim 20, wherein the crosslinking agent is carboxymethyl cellulose.

22. The method of claim 1, wherein the crosslinking agent effects a complete cure of the collagen or gelatin in about 30 seconds to 24 hours.

23. The method of claim 22, wherein the crosslinking agent effects a complete cure of the collagen or gelatin in less than 5 minutes.

24. The method of claim 1, wherein the crosslinking agent is present in an amount of about 0.01-35%.

25. The method of claim 24, wherein the crosslinking agent is present in an amount of about 0.1-5%.

26. The method of claim 25, wherein the crosslinking agent is present in an amount of about 0.6-1.2%.

27. The method of claim 1, wherein the sealant further comprises a second crosslinking agent.

28. The method of claim 27, wherein the second crosslinking agent cures the collagen or gelatin in about 30 seconds to about 24 hours.

29. The method of claim 28, wherein the second crosslinking agent cures the collagen or gelatin in less than 5 minutes.

30. The method of claim 27, wherein the second crosslinking agent is an aldehyde.

31. The method of claim 30, wherein the second crosslinking agent is glutaraldehyde.

32. The method of claim 27, wherein the second crosslinking agent is an epoxy compound.

33. The method of claim 32, wherein the epoxy compound is a polymer with 2 or more pendant epoxy groups.

34. The method of claim 33, wherein the epoxy compound is a polyethylene glycol or diglycidyl ether.

35. A method of preventing post-operative adhesions between healing tissues during the healing process comprising:

a) mixing a solution or suspension of a proteinaceous material with a solution of a protein crosslinking agent at an elevated temperature to form a flowable sealant;

b) introducing said flowable sealant into a surgical site either during surgery or post-operatively, said sealant forming a continuous layer on the tissue; and

c) allowing the layer to continue crosslinking on the tissue to form a cured biocompatible layer of sealant that acts as a barrier, adheres to the tissue, prevents adhesion of the healing tissues and that degrades in about 2 to about 120 days.

36. The method of claim 35, wherein the cured layer of sealant degrades in at least about 2 -90 days.

37. The method of claim 36, wherein the cured layer of sealant degrades in at least about 2-30 days.

38. The method of claim 35, wherein the solution or suspension of proteinaceous material is collagen or gelatin.

39. The method of claim 38, wherein the solution or suspension consists of about 5% to about 60% collagen or gelatin.

40. The method of claim 39, wherein the solution or suspension consists of about 30% to about 55% collagen or gelatin.

41. The method of claim 40, wherein the solution or suspension consists of about 40% to about 50% collagen or gelatin.

42. The method of claim 38, wherein the solution or suspension is about 10% to about 40% collagen or gelatin and the sealant is applied to the tissue by spraying.

43. The method of claim 42, wherein the solution or suspension is about 25% to about 35% collagen or gelatin and the sealant is applied to the tissue by spraying.

44. The method of claim 35, wherein the sealant further comprises a plasticizer.

45. The method of claim 44, wherein the plasticizer is polyethylene glycol having a molecular weight ranging from about 200 to about 1000 Daltons.

46. The method of claim 45, wherein the plasticizer is polyethylene glycol having a molecular weight of about 400 Daltons.

47. The method of claim 44, wherein the plasticizer is present in an amount of about 1-30%.

48. The method of claim 47, wherein the plasticizer is present in an amount of about 5-10%.

49. The method of claim 35, wherein the crosslinking agent is an aldehyde.

50. The method of claim 49, wherein the aldehyde is a di- or polyaldehyde.

51. The method of claim 50, wherein the di- or polyaldehyde is glutaraldehyde, glyoxal, or dialdehyde starch.

52. The method of claim 35, wherein the crosslinking agent is an oxidized carbohydrate.

53. The method of claim 52, wherein the oxidized carbohydrate is malto-dextrin.

54. The method of claim 35, wherein the crosslinking agent is a polysaccharide.

55. The method of claim 54, wherein the crosslinking agent is carboxymethyl cellulose.

56. The method of claim 35, wherein the crosslinking agent effects a complete cure of the collagen or gelatin in about 30 seconds to 24 hours.

57. The method of claim 56, wherein the crosslinking agent effects a complete cure of the collagen or gelatin in less than 5 minutes.

58. The method of claim 35, wherein the crosslinking agent is present in an amount of about 0.01-35%.

59. The method of claim 58, wherein the crosslinking agent is present in an amount of about 0.1-5%.

60. The method of claim 59, wherein the crosslinking agent is present in an amount of about 0.6-1.2%.

61. The method of claim 35, wherein the sealant further comprises a second crosslinking agent.

62. The method of claim 61, wherein the second crosslinking agent cures the collagen or gelatin in about 30 seconds to about 24 hours.

63. The method of claim 62, wherein the second crosslinking agent cures the collagen or gelatin in less than 5 minutes.

64. The method of claim 61, wherein the second crosslinking agent is an aldehyde.

65. The method of claim 64, wherein the second crosslinking agent is glutaraldehyde.

66. The method of claim 61, wherein the second crosslinking agent is an epoxy compound.

67. The method of claim 66, wherein the epoxy compound is a polymer with 2 or more pendant epoxy groups.

68. The method of claim 67, wherein the epoxy compound is a polyethylene glycol or diglycidyl ether.

69. A biocompatible composition for sealing tissue and for preventing post-surgical adhesions comprising:

a) a proteinaceous material component; and

b) a protein crosslinking component which promotes crosslinking of the protein component in a);

wherein the composition exhibits a peel bond strength of at least about 3 N/m.

70. The composition of claim 69, wherein the proteinaceous material component is collagen or gelatin.

71. The composition of claim 70, wherein the proteinaceous material component consists of about 5% to about 60% collagen or gelatin.

72. The composition of claim 71, wherein the proteinaceous material component consists of about 30% to about 55% collagen or gelatin.

73. The composition of claim 72, wherein the proteinaceous material component consists of about 40% to about 50% collagen or gelatin.

74. The composition of claim 70, wherein the proteinaceous material component consists of about 10% to about 40% collagen or gelatin and is applied to the tissue by spraying.

75. The composition of claim 74, wherein the protein component consists of about 25-35% collagen or gelatin.

76. The composition of claim 59, wherein the composition degrades in at least about 2-120 days.

77. The composition of claim 69, wherein the composition degrades in at least about 2-120 days.

78. The composition of claim 77, wherein the composition degrades in at least about 2-90 days.

79. The composition of claim 78, wherein the composition degrades in at least about 2-30 days.

80. The composition of claim 69, wherein the composition further comprises a plasticizer.

81. The composition of claim 80, wherein the plasticizer is polyethylene glycol having a molecular weight ranging from about 200 to about 1000 Daltons.

82. The composition of claim 81, wherein the plasticizer is polyethylene glycol having a molecular weight of about 400 Daltons.

83. The composition of claim 80, wherein the plasticizer is present in an amount of about 1-30%.

84. The composition of claim 83, wherein the plasticizer is present in an amount of about 5-10%.

85. The composition of claim 69, wherein the crosslinking agent is an aldehyde.

86. The composition of claim 85, wherein the aldehyde is a di- or polyaldehyde.

87. The composition of claim 86, wherein the di- or polyaldehyde is glutaraldehyde, glyoxal, or dialdehyde starch.

88. The composition of claim 69, wherein the crosslinking agent is an oxidized carbohydrate.

89. The composition of claim 88, wherein the oxidized carbohydrate is malto-dextrin.

90. The composition of claim 69, wherein the crosslinking agent is a polysaccharide.

91. The composition of claim 90, wherein the crosslinking agent is carboxymethyl cellulose.

92. The composition of claim 69, wherein the crosslinking agent effects a complete cure of the collagen or gelatin in about 30 seconds to 24 hours.

93. The composition of claim 92, wherein the crosslinking agent effects a complete cure of the collagen or gelatin in less than 5 minutes.

94. The composition of claim 69, wherein the sealant further comprises a second crosslinking agent.

95. The composition of claim 94, wherein the second crosslinking agent cures the collagen or gelatin in about 30 seconds to about 24 hours.

96. The composition of claim 95, wherein the second crosslinking agent cures the collagen or gelatin in less than 5 minutes.

97. The composition of claim 94, wherein the second crosslinking agent is an aldehyde.

98. The composition of claim 97, wherein the second crosslinking agent is glutaraldehyde.

99. The composition of claim 94, wherein the second crosslinking agent is an epoxy compound.

100. The composition of claim 99, wherein the epoxy compound is a polymer with 2 or more pendant epoxy groups.

101. The composition of claim 100, wherein the epoxy compound is a polyethylene glycol or diglycidyl ether.

102. The composition of claim 94, wherein the second crosslinking agent is a polysaccharide.

103. The composition of claim 102, wherein the second crosslinking agent is carboxymethyl cellulose.

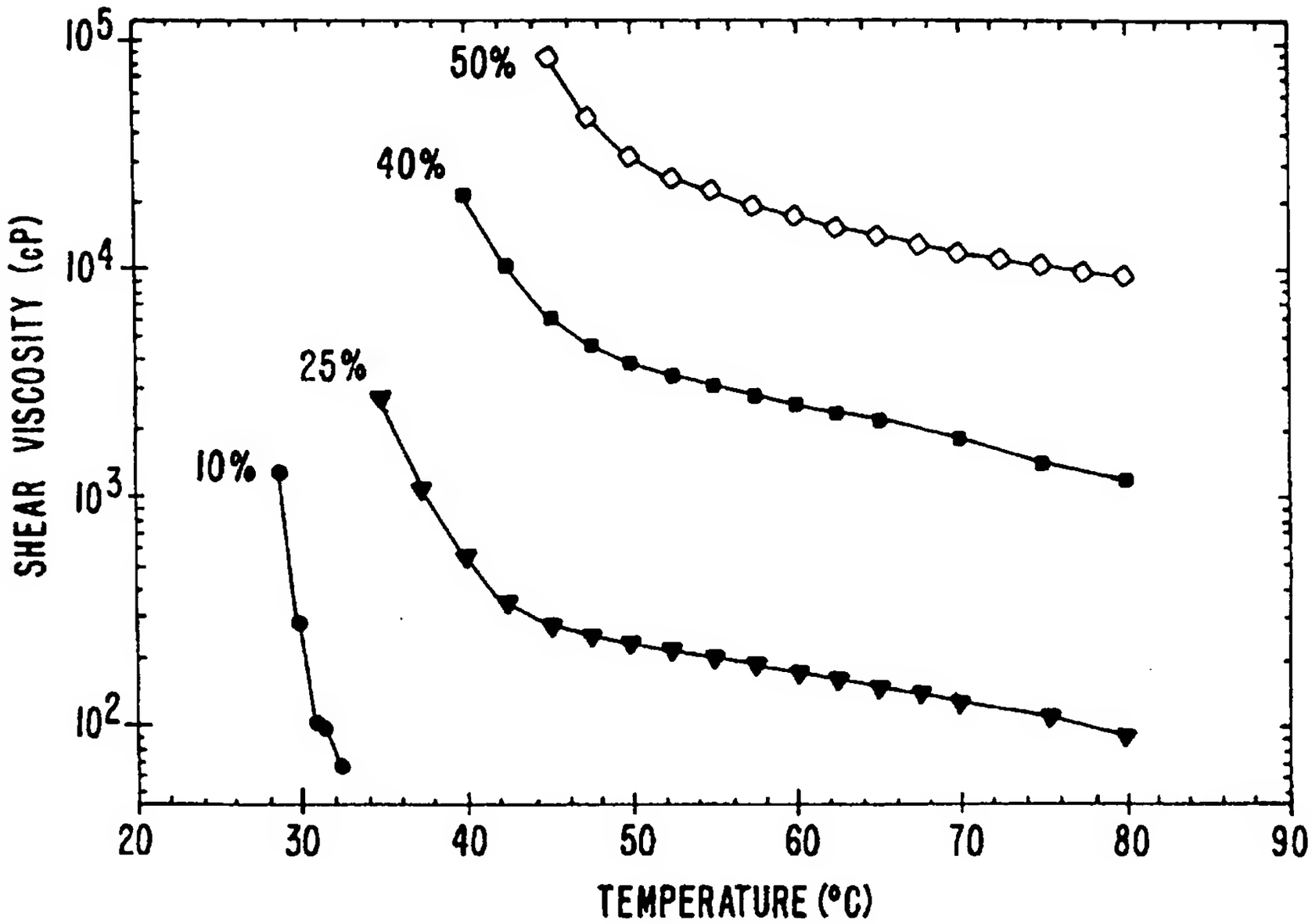
SHEAR VISCOSITY (cP)
10^5
10^4
10^3
10^2
50%
40%
25%
10%
20
30
40
50
60
70
80
90
TEMPERATURE (°C)

FIG. 1.

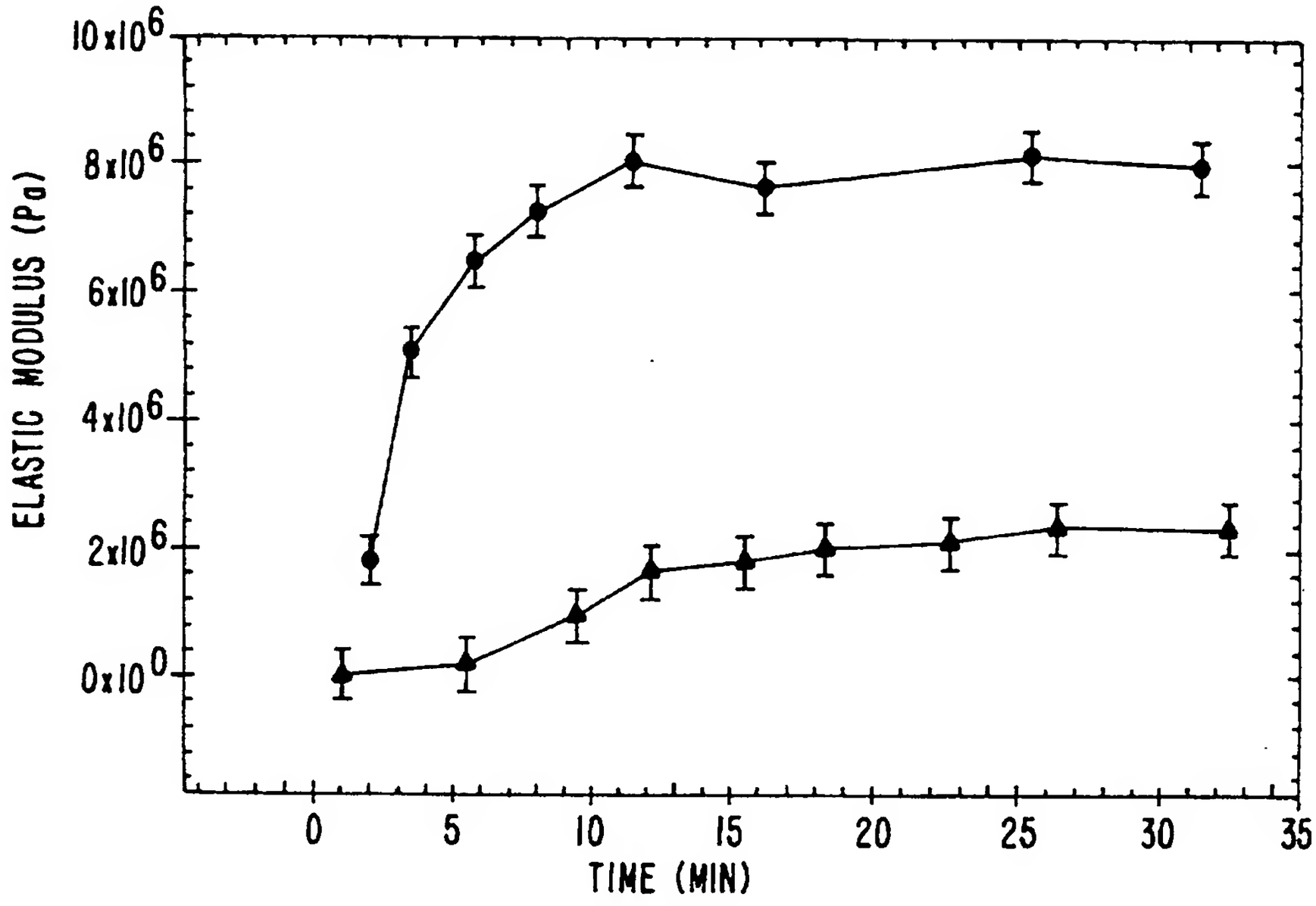
10x10^6
8x10^6
6x10^6
4x10^6
2x10^6
0x10^0
ELASTIC MODULUS (Pa)
0
5
10
15
20
25
30
35
TIME (MIN)
50% GELATIN & DIALDEHYDE STARCH GLUE
50% GELATIN & WATER

FIG. 2.

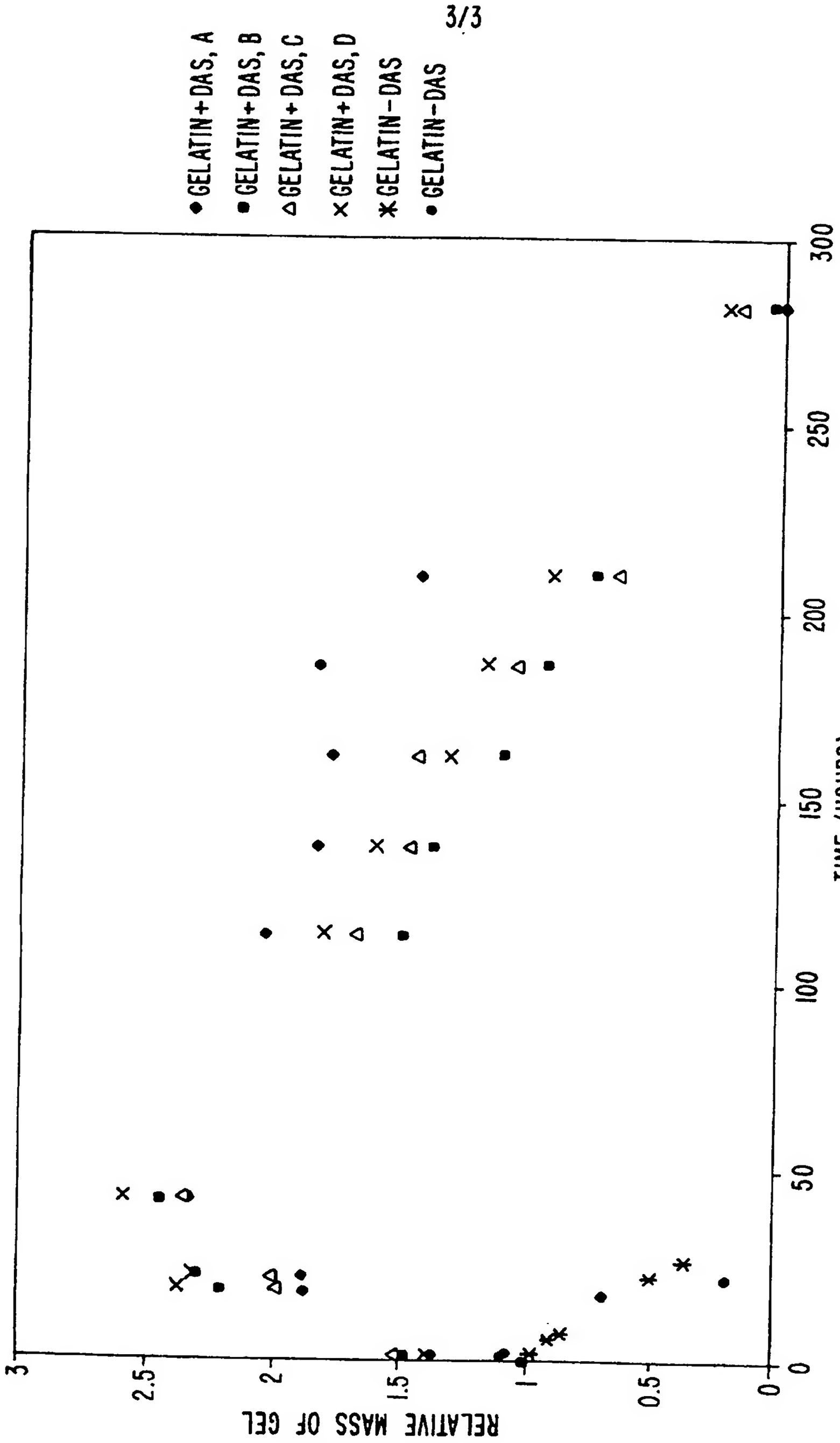
● GELATIN+DAS, A
■ GELATIN+DAS, B
△ GELATIN+DAS, C
× GELATIN+DAS, D
✱ GELATIN-DAS
● GELATIN-DAS
RELATIVE MASS OF GEL
3
2.5
2
1.5
1
0.5
0
0
50
100
150
200
250
300
TIME (HOURS)

FIG. 3.

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US97/02542

A. CLASSIFICATION OF SUBJECT MATTER

IPC(6) : A61F 2/02; A61B 17/04
US CL : 424/426; 606/229, 230

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

U.S. : 424/426; 606/229, 230

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

Please See Extra Sheet.

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	Abstract, Abstract number 111:45215, MIYATA ET AL. Biodegradable Antiadhesive Membrane, Jinko Zoki (1989), 18(1), 93-6.	1-102

☐ Further documents are listed in the continuation of Box C. ☐ See patent family annex.

*	Special categories of cited documents:	"T"	later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
"A"	document defining the general state of the art which is not considered to be of particular relevance	"X"	document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
"E"	earlier document published on or after the international filing date	"Y"	document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
"L"	document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)	"&"	document member of the same patent family
"O"	document referring to an oral disclosure, use, exhibition or other means		
"P"	document published prior to the international filing date but later than the priority date claimed		

Date of the actual completion of the international search 01 MAY 1997	Date of mailing of the international search report 09 JUN 1997
Name and mailing address of the ISA/US Commissioner of Patents and Trademarks Box PCT Washington, D.C. 20231 Facsimile No. (703) 305-3230	Authorized officer Carlos Azpuru Telephone No. (703) 308-2351

Form PCT/ISA/210 (second sheet)(July 1992)★

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US97/02542

B. FIELDS SEARCHED
Electronic data bases consulted (Name of data base and where practicable terms used):

CAS ONLINE: s wound(w)heal? or suture
s L1 and (protein? or fibrin or fibrinogen or thrombin or albumin or Factor(w)VIII or elastin or gelatin)
s L2 and (crosslink? or cross(w)link?)
s L3 and (aldehyde# or polyaldehyde# or glutaraldehyde# or glyoxal or dialdehyde(w)starch or malto(w)dextrin# or oxidized(w)carbohydrate# or epoxy or polyethylene(w)glycol(w)ether or diglycidyl(w)ether)
sL4 and (plasticizer# or polyethylene(w)glycol)